

Label-Free/Live Adherent Cell Confluence

Automated Assessment of the Area Occupied by Unlabelled Living CHO Cells in 96 well plates using the MIAS[®]-2 truVIEW Microscopy Reader and eaZYX[®] Imaging Software

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Objective

In this application note, an automated method for detection of the area occupied by unlabelled, adherent and living CHO cells in 96-well plates, using the MIAS[®]-2 truVIEW microscopy reader (figure 1), is described.

Automated whole-well image capture and image analysis using eaZYX[®] imaging software showed accurate detection of the area covered by CHO cells from seeding densities ranging from 2000 to 24000 cells per well, corresponding with 10% and 70% confluence, respectively.

Introduction

The MIAS-2 is a compact fully automated versatile microscopy reader combining 5 brightfield with up to 8 fluorescent modes. For this application the brightfield mode is selected. Using cell seeding experiments with the adherent cell line CHO as an example, this application note describes how the detection of the area covered by the cells in plates can be executed fully automated and in a fully non-invasive manner at plate cycle times that match high content screening requirements.

Materials/Methods

CHO cells (ATCC CCL-61) were grown in Ham's F12 medium (Invitrogen, Cat.No. 21765-029) in 175 cm² T-flasks. The density of the cell suspension was calibrated using a Neubauer counting chamber immediately prior to cell seeding in plates (1). 2000 to 28000 CHO cells were seeded in 200 µl medium into 96 well plates (Greiner, CellStar, Cat.No. 655180). The plates were incubated overnight in the incubator (95% RH, 37 °C, 5% CO₂) allowing cells to adhere to, and to spread at the bottom of the well. Prior to scanning with MIAS-2, plates were equilibrated to room temperature for 15 minutes. Whole well, 5 x 5 tiled images were captured using a 5x objective and 1.0x Optovar settings. Brightfield light settings were adjusted for proper illumination at the centre of the wells. A specific confluence application for adherent cells was selected for analysis. Whole well overviews with image analysis result overlays were created using eaZYX-imaging software (2). Data tables were imported in a spreadsheet program (MS-Excel) and graphics were prepared. The images shown in figures were JPEG compressed and contrast adjusted prior publication.

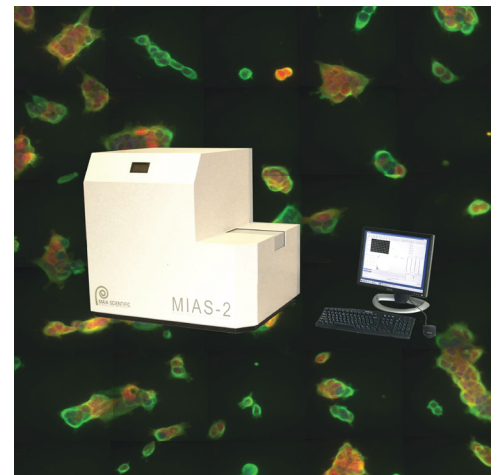


Figure 1. The MIAS-2 reader equipped with eaZYX IMAGING software.

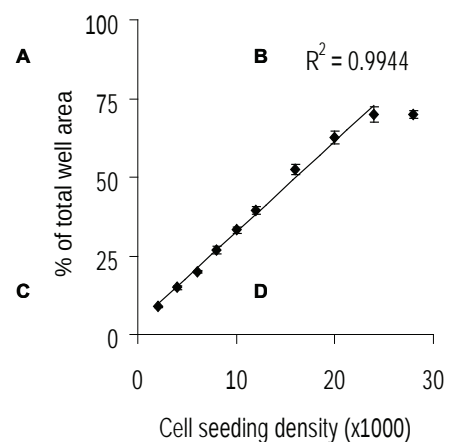


Figure 2. Graphical presentation of the percentage of the total well area versus the actual cell seeding density (n=6). Linear regression analysis results in a correlation coefficient of 0.9944.

